Mass Spectrometry Vapor Analysis for Improving Explosives Detection Canine Proficiency

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ABSTRACT: Canines remain the gold standard for explosives detection in many situations, and there is an ongoing desire for them to perform at the highest level. This goal requires canine training to be approached similarly as scientific sensor design. Developing a canine training regimens is made challenging by a lack of understanding of the canine's odor environment, which is dynamic and typically contains multiple odorants. Existing methodology assumes that the handler's intention is an adequate surrogate for actual knowledge of the odors cuing the canine, but canines are easily exposed to unintentional explosive odors through training material cross-contamination. A sensitive, real-time (~1 sec) vapor analysis mass spectrometer was developed to provide tools, techniques, and knowledge to better understand, train, and utilize canines. The instrument has a detection library of nine explosives and explosive-related materials consisting of 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 2,4,6-trinitrotoluene (TNT), nitroglycerin (NG), 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), penta erythritol tetranitrate (PETN), triacetone triperoxide (TATP), hexamethylene triperoxide diamine (HMTD), and cyclohexanone, with detection limits in the part-per-trillion to part-per-quadrillion range by volume. The instrument can illustrate aspects of vapor plume dynamics, such as detecting plume filaments at a distance. The instrument was deployed to support canine training in the field, detecting cross-contamination among training materials, and developing an evaluation method based on the odor environment. Support for training material production and handling was provided by studying the dynamic headspace of a non-explosive HMTD training aid that is in development. These results supported existing canine training and identified certain areas that may be improved.

INTRODUCTION:

Canines can detect many explosives even in environments that contain multiple odorants, making them the gold standard for explosive detection in many situations. However, while they are generally effective, large performance discrepancies (40% or more) may be observed between canines.[1-5] Even though some differences can be attributed to biological variations,[3] other sources of variation likely come from training and testing deficiencies. There are currently multiple canine performance standards, such as the National Odor Recognition Testing (NORT) and the Scientific Working Group on Dog and Orthogonal detector Guidelines (SWGDOG), and procedural variations exist between agencies.[6]

Beyond policy differences, training efficacy is hampered by difficulties quantifying true performance. Canines are currently judged during training and testing by how well they find intentionally hidden explosives. This method makes intuitive sense because handlers cannot see odors, so their intention is used as a surrogate for whether or not an odor is present. However, unintentional explosive odors can be presented if cross-contaminated training materials are used. Multiple explosive materials are often used during training, producing a situation where cross-contamination has an opportunity to occur. Training materials may also be mixtures that contain energetics with variable vapor pressure,[7] and susceptibility to degradation.[8-10] Measuring the canine's odor environment is therefore an important part of evaluating canine performance and optimizing training. For canine scent detection to achieve its optimal analytical merits in these complex environments will require adapting not only the best practices in animal training, but also sensor development.[11]

There are many techniques available for trace explosive detection.[12-15] Mass spectrometry (MS) is well-suited for characterizing the canine's odor environment because of its sensitivity and broadband chemical coverage.[12,15] Methods such as solid-phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS), which combine gas sampling, chromatographic separation, and MS, have already been used for analyzing canine training materials.[12,16,17] However, SPME collection biases can distort results, and sensitivity is often limited with GC-MS instrumentation. Long sampling periods also produce time-averaged data that do not reflect a dynamic vapor environment.[18] MS vapor analysis techniques that do not require extensive sampling and separation times for explosives and other compounds have also been developed.[12] Ionization methods include secondary electrospray ionization (SESI),[8,19-21] dielectric barrier discharge ionization (DBDI),[21,22] selected-ion-flow-tube (SIFT),[23,24] and proton transfer reaction (PTR),[25,26] among others.[12,27,28] These techniques often achieve parts-per-trillion (ppt) or lower levels of detection sensitivity,[19,21,27] performances that may be comparable to or better than canines.[1]

Given the challenges of canine training and the advances in analytical technology, perhaps one implicit focus of the explosive detection field has been to develop instruments to copy and replace canine functionality. For example, SESI-MS has been used for screening cargoes, and vapor screening portals

have been designed.[29] But for several reasons, including logistical ones such as cost and maintenance, these instruments are not yet widely utilized. We believe that a more immediate and impactful application is to improve the performance of already broadly deployed canine teams. Improvement areas include improving training material production, monitoring cross-contamination between canine training materials, and developing new canine evaluation methods. The additional rigor, in a way treating canine teams as chemical sensors in addition to as service teams, will improve training efficiency and operational performance.

In support of canine training, the Department of Homeland Security Science and Technology Directorate's Explosives Detection Canine Program supported development of an MS instrument with a dual SESI and DBDI source. SESI crosses the analyte vapor stream with an electrospray,[20] and DBDI passes the sample through a plasma or reacts the analyte with reactive plasma species.[30] The instrument can reveal, in real-time, aspects of vapor behavior, such as plume filaments and exfiltration points. The instrument was used to improve canine training in several areas. Training exercises were held with two in-service canine teams. Cross-contamination among training materials was detected, and a new evaluation metric was developed based on the canine's odor environment rather than the handler's intention. The instrument was also used to help develop a non-explosive hexamethylene triperoxide diamine (HMTD) training aid. Non-explosive training aids are materials made by dispersing small quantities of energetics onto an inert substrate, producing vapor signatures while remaining safe to train with in the operational environment. HMTD training aid production is challenging because HMTD degrades easily.[8-10] The labile nature has even made studying HMTD properties difficult. However, Steinkamp et al. have investigated how storage conditions and sample clean up can affect HMTD degradation,[10] and Aernecke et al. estimated the vapor pressure of HMTD using surrogate materials.[8] The dynamic headspace composition of HMTD training aids were measured to provide feedback on the aid production, storage, and deployment processes.

EXPERIMENTAL

MATERIALS:

LC/MS grade methanol was purchased from VWR (Radnor, PA, USA). Energetics standards were Cerilliant certified standards (Round Rock, TX, USA) purchased from Sigma Aldrich (St. Louis, MO, USA). Standards included 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), nitroglycerin (NG), pentaerythritol tetranitrate (PETN), 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), and 2,4,6-trinitrotoluene (TNT). Acetic acid, cyclohexanone, and 2,4-dinitrotoluene (97%) were also purchased from Sigma Aldrich. Ammonium hydroxide (30%) was purchased from J.T. Baker (Center Valley, PA, USA).

Peroxide energetics (HMTD and TATP) were synthesized in house using published procedures.[31,32] Approximately 600 mg of material were made at a time, and 40-100 mg portions were used for normalization and as the benchmark HMTD to compare against the training aids. Material purity was measured to be >99% via ^{1}H- and ^{13}C-NMR.

Safety note: HMTD and TATP are most hazardous after they are purified and dried. Our standard operating procedure was to never prepare batches greater than ~500 mg and to never fully dry quantities more than ~200 mg. Dried HMTD and TATP were stored at <4 °C and handled only with wooden or Teflon® spatulas to prevent static discharge. Using these precautions, we never experienced an accidental detonation.

All HMTD training aids were provided by the John Hopkins University Applied Physics Laboratory (JHU-APL). Training aids were stored in glass vials or metal mesh tins wrapped in aluminum foil, and some were further sealed in anti-static bags until analysis.

Bulk charges (ammonium dynamite, C4, detonation cord, Semtex-A, smokeless powder, and TNT) were provided by the Hanscom Airforce Base (HAFB) 66th Security Forces Squadron (SFS) Canine Team. Because the exact amount of explosives used for canine training is considered sensitive information, we will withhold the mass of each bulk charge. These charges are mixtures with many components, and the expected energetics in each charge are listed in Table S1. Bulk charges were handled only by qualified personnel from the HAFB 66th SFS.

MASS SPECTROMETRY AND IONIZATION SOURCE:

All mass spectrometry measurements were performed on a Sciex 5500 QTRAP mass spectrometer (Sciex; Concord, Ontario, Canada) equipped with Analyst 1.6 build 3773. Custom secondary electrospray ionization (SESI) and dielectric barrier discharge ionization (DBDI) sources were used for vapor ionization. Source temperature was held at 100 °C for most operation. N_2 was provided using a nitrogen generator (ABN2ZA; Peak Scientific; Inchinnan, UK). Q1 scans (m/z 40-350 or m/z 40-400) were taken with 20 V declustering potential (DP) and 10 V entrance potential (EP). Multiple reaction monitoring (MRM) scans were taken for several energetics and cyclohexanone. Instrument parameters in MRM mode (e.g. declustering potential) were optimized for each transition. To make the system deployable to canine training sites, the system was placed on a stainless steel cart that include auxiliary equipment such as vacuum pumps, ion source power supplies, and N_2 generator (Figure S1).

Technical diagrams of our SESI and DBDI sources are shown in Figure S2. For SESI, two ESI needles (Agilent; Santa Clara, CA, USA) flowing separate solvents were used for positive (70:30 methanol: water + 2% acetic acid) and negative (70:30 methanol: water + 0.03% ammonium hydroxide) mode analysis. ESI spray was maintained at ±4.5 kV, and 1 L/min nebulizer flow. Solvent flow (10 - 20 µL/min) was maintained using a Series 200 HPLC pump (PerkinElmer; Waltham, MA, USA) or an LD Class HPLC pump (Scientific Systems; State College, PA). Additional N_2 gas flow and vacuum pumping was used to achieve a 1 L/min sampling flow rate into the ionization source.

For DBDI, plasma generation was achieved using a PVM500 power supply (Information Unlimited; Amherst, NH, USA) to supply an AC pulse (±7.8 kV Vpp, 30 kHz) of 760 µs in a 19.76 ms cycle. The source was composed of an outer copper electrode (id: 8.6 mm, od: 11.2 mm) held at ground, an inner stainless steel electrode (id: 3.2 mm, od: 6.4 mm), and a Teflon dielectric (length: 31.8 mm, id: 6.4 mm, od: 8.6 mm). N_2 was supplied to the source at 1.5 L/min. The source was connected 12" from the mass spectrometer inlet using a 0.5" ID stainless steel tube and positioned perpendicularly from the sample flow path using a tee. Flow through the 0.5" stainless steel tubing was held at 2.5-4 L/min for typical operation.

SINGLE-POINT EXTERNAL CALIBRATION:

Single-point external calibration was used to semi-quantify several energetics. Based on previous protocols,[8,33] vapor generators were made by depositing several hundred micrograms of an energetic on glass wool in a glass tube (id: 3.9 mm, length: 7 cm). Analysis was conducted in MRM mode, with 60 s of background (no vapor generator) followed by 60 s in which N_2 was flowed through the generator into the ionization source. Based on the energetic's vapor pressure,[7] the flow rate was between 20-1000 mL/min and was chosen to avoid saturating instrument response. The limit of detection (LOD) for each energetic was estimated by calculating the vapor concentration that would produce a signal-to-noise ratio (S/N) of 3. Care was taken during LOD calculation to dilute the amount of energetic sent into the ionization source based on the intake flow rate (1 L/min for SESI and 2.5-4 L/min for DBDI, see section on Mass Spectrometry and Ionization Source). Vapor generators were used to quantify TNT, 2,4-DNT, 2,6-DNT, HMTD, NG, RDX, and PETN. Data for TATP and cyclohexanone were produced by placing a vial of material ~1 mm below the source intake.

PLUME DETECTION:

Several experiments were conducted to demonstrate aspects of vapor plume behavior. 1) To show vapor travel via filaments, a vial of TATP was placed 30 cm from the ionization source. Background (5 min) was taken before and after the vial was placed and removed. 2) To show high vapor concentration even from trace quantities, 2 μg of TNT was dropcasted onto aluminum foil and passed in front of the source inlet, starting from ~30 cm away. The drop-casted spot was ~1 cm underneath the sample inlet at the closest point. Analysis lasted 60 s, with the TNT spot directly in front of the inlet at 30 s. 3) To show odor exfiltration points, a brick of C4 with a hole in its plastic wrapper was analyzed.

CANINE TRAINING MATERIALS ANALYSIS:

Bulk charges and ammunition cans from the HAFB 66th SFS (see "Materials") were analyzed via SESI in Q1 and MRM modes. C4, detonation cord, ammonium dynamite, and Semtex-A were additionally analyzed using DBDI in MRM mode. Trace energetics (micrograms or less) were analyzed using SESI in Q1 and MRM modes, and DBDI in MRM mode. In most cases, 30 - 60 s of background was taken before placing the material ~10 mm in front of the source inlet.

CANINE TRAINING:

Two canines from the HAFB 66th SFS, and two canines from the Massachusetts Bay Transportation Authority (MBTA) participated in the experiment. All canines were handled by their dedicated handlers.

Canines were tasked to find materials including bulk charges from the HAFB 66th SFS, fingerprints of energetics, dropcast spots of energetics (fingerprint-level quantity), and 500 μL of cyclohexanone in a GC vial punctured with a 22-gauge needle. Some materials were randomly hidden, requiring the canine to search through a room, while others were placed in a circle among blanks. Some materials were placed in containers before being hidden, such as a paint can covered with a lid drilled with a 0.75" hole, a Ray Allen canine training can (Colorado Springs, CO, USA), or Ziploc bags and envelopes. All handlers were kept blind to the hide locations.

Bulk charges, trace energetics, cyclohexanone, and blanks falsely identified by canines were analyzed via MS. Care was taken to maintain the same degree of odor concealment during MS analysis as during canine testing (Figure S1).

HMTD TRAINING AID MEASUREMENT AND CALIBRATION:

HMTD training aids were measured via SESI as soon as possible after removal from packaging. Aids were placed in a Teflon beaker under the SESI source inlet. Based on previous work, analysis covered HMTD and its associated degradation and starting materials.[8,10] Compounds were monitored in positive and negative polarities (Figure S3). HMTD, trimethylamine (TMA), dimethyl formamide (DMF), and hexamine (HEX) were analyzed in positive polarity. Formic acid (FA), and N-formyl formamide (NFF) were analyzed in negative polarity. Data collection manually alternated between positive and negative polarities every five min, starting with the positive polarity. Total analysis time was two hours, representing alternating 12 positive- and 12 negative-polarity measurements. The first two five-min periods were background measurements in each polarity. In the third five min period, one min was allowed to pass before the training aid was introduced. The training aid remained in place until the penultimate five min period, which was taken in positive polarity, and the aid was removed one min into this period in order to observe the background after aid removal. The last five min period was taken in negative polarity and was a background. Several samples of pure HMTD were measured in the same way.

STATISTICAL COMPARISON OF HMTD TRAINING AIDS:

Data collected each day were normalized against a single-point HMTD external calibration point measured every day. Samples were further grouped together based on the time period when they were measured (typically weeks at a time) and normalized against bulk synthetic HMTD samples measured at around the same time frame (see "Materials").

Training aid quality was scored by calculating the Euclidean distance between each aid and pure HMTD. The median intensities of the six monitored headspace compounds at the beginning and the end of the analysis were included in the calculation (MRM transitions are HMTD: 209/88; DMF: 74/46; TMA: 60/44; HEX: 141/42; FA: 91/45; NFF: 72/42), making the score a 12-dimentional Euclidean distance (six compounds at two time points). For compounds monitored in positive polarity, the median intensities at the 3rd and 21st five min periods were used (excluding the first minute in the 3rd period, which was before aid introduction). For negative polarity compounds, the median intensities at the 4th and 22nd five min periods were used. For the extracted median values from pure HMTD controls, the median for all replicate measurements was further taken.

RESULTS AND DISCUSSION

Instrument and Detection Library Development

Because our aim is to support canine training, it is important to develop a mass spectrometer with a large enough detection library to cover the many training materials. Analysis should also be conducted without perturbing the sample with a probe (e.g. heating, spray, or laser) in order to reproduce how canines may perceive a training material. The necessary explosives detection library extends beyond what is often demonstrated for real-time, ambient vapor MS analysis.[19,23,25-28] To detect a large range of explosives, our mass spectrometer is equipped with two ionization sources: SESI and DBDI (Figure S2 in Supporting Information). The SESI source ionizes many materials with good performance (Table 1),[19,21] and the DBDI assembly is used for detecting lower vapor pressure explosives like RDX and PETN. The DBDI assembly is incorporated into a larger set up based on the work done by Ewing et al., which relies on forming nitrate adduct ions by interacting the analyte with reactive species produced from a corona.[27] The DBDI assembly is positioned perpendicularly to the analyte vapor stream (Figure S2(C)). The plasma, containing nitrates, is directed to interact with the analyte through a tee. The sample does not pass through the plasma, allowing plasma characteristics to stay constant during analysis. While sensitive for RDX and PETN, our DBDI set up cannot detect compounds that do not readily form nitrate adducts. The SESI and DBDI sources are therefore complementary. Our system is unique in possessing both SESI and DBDI sources that can be quickly swapped (a few minutes) to cover a large range of analytes.

Our MRM detection library includes multiple energetics, covering those that preferentially ionize via SESI or DBDI and in positive and negative polarities. The explosives include disparate analyte classes, such as nitramines (RDX), nitrate esters (PETN), nitroaromatics (TNT, DNT), and peroxides (HMTD and TATP). If necessary, we may detect all nine compounds by switching between positive and negative polarities and between SESI and DBDI ionization. For tracking cross-contamination between training materials, we often monitor many of these energetics per experiment. LOD for each compound is roughly estimated using single-point external calibration (Table 1), which are typically at the ppt to parts-per-quadrillion (ppq) level, hundreds of times or more below the saturated partial vapor pressure (P_o) for many energetics,[7] and comparable to or better than canine capability.[1] Defining LOD as a fraction of P_o is a simple metric for estimating performance for in-field situations, in which dilution and concealment can lower the vapor pressure by several orders of magnitude below the saturated partial vapor pressure.[34] Of note, we also believe our LODs for TATP and cyclohexanone are lower than described in Table 1. These two materials have high vapor pressures compared to the other energetics and would have been difficult to incorporate into our vapor generator set up. They were therefore analyzed by placing a vial of material ~1 mm beneath the ionization inlet. We were able to obtain signal for both materials, but transfer efficiency into the source may be poor. Instrument response also saturated when analyzing cyclohexanone. These factors lead us to believe that the LODs in Table 1 are conservative estimates for TATP and cyclohexanone.

Plume Dynamics and Explosives Detection

One strength of the instrument is its real-time detection capability, which was demonstrated previously by Staymates et al.[35] in a study that uses a canine nose-inspired sample inlet to increase the instrument's aerodynamic reach.[35] Real-time response is a consideration for security portal design,[29] and instruments such as the one developed here may inform criteria for low-cost sensors or even itself serve as the screening technology.[36] For canine training, rapid response to vapor signatures is especially important. A canine's odor environment is not composed of static, diffused vapors, but rather of dynamic plumes that shift spatially and temporally. This is particularly important when a canine is searching for threats concealed on individuals moving about in a dynamic environment. Understanding plume behavior is therefore an important part of effective canine deployment. Our instrument is used to show many aspects of vapor plume dynamics to improve handler knowledge, such as 1) plume filaments, 2) production of concentrated odors from trace material quantities, and 3) odor exfiltration points from concealed explosives.

Table 1. SESI and DBDI detection limits for explosives and explosive-related materials

Compound (Polarity)	Precursor Ion	MRM	LOD (ppt)	P/P$_o$
SESI				
2,4-DNT (-)	M-H	181/46	4×10^{-1}	2×10^{-6}
	M-H	181/135	1×10^{0}	4×10^{-6}
2,6-DNT (-)	M-H	181/46	3×10^{2}	6×10^{-4}
	M-H	181/135	2×10^{4}	4×10^{-2}
HMTD (+)	M+H	209/88	6×10^{-2}	1×10^{-3}
NG (-)	M+HCO$_3$ M+CH$_3$NO$_2$	288/46	2×10^{1}	5×10^{-5}
	M+75	302/46	2×10^{2}	4×10^{-4}
TNT (-)	M-H	226/46	6×10^{-1}	2×10^{-4}
	M-H	226/76	1×10^{0}	2×10^{-4}
TATP[a] (+)	C$_3$H$_7$O$_3$	91/43	$<1 \times 10^{4}$	$<1 \times 10^{-3}$
	M+NH$_4$	240/74	$<2 \times 10^{4}$	$<1 \times 10^{-3}$
Cyclohexanone[a] (+)	Dimer	197/81	$<3 \times 10^{7}$	$<1 \times 10^{-2}$
	Dimer	197/99	$<3 \times 10^{6}$	$<7 \times 10^{-4}$
DBDI				
Compound (Polarity)	Precursor Ion	MRM	LOD (ppt)	P/P$_o$
RDX (-)	M+NO$_3$	284/62	4×10^{-2}	1×10^{-2}
PETN (-)	M+NO$_3$	378/62	5×10^{-1}	9×10^{-2}

P_o = Saturated partial vapor pressure[7]

[a] Estimated by placing a vial ~1 mm under the ionization source inlet

Detecting Vapor Plume Filaments. Vapors travel via both convection and diffusion, but on the length scales important in these experiments, convective transport is the dominant mechanism, leading to formation of vapor filaments sweeping over the surrounding area.[37-39] There has been evidence that scent-detecting animals determine an odor source based on how frequently a vapor plume filament is encountered.[38] To help canine handlers visualize plume filaments, we placed a vial of TATP 30 cm from the instrument. TATP is a homemade explosive used in the 2015 and 2016 Paris and Brussels attacks.

Signal was observed as a series of spikes (~1 s in duration), with each spike representing an instance when the plume filament passed over the instrument (Figure 1(A)). The average signal over the ten minute period is ~200 ppb and is shown as a dotted line, but the concentration in a spike is higher (> 1 ppm). For canine training purposes, it shows that canines are interacting in a dynamic environment that has periods of strong scents, rather than a static one with constant, low vapor concentrations. From an analysis standpoint, rapid response can also be a way to improve sensitivity by taking advantage of the high concentration in a filament.

Concentrated Odor From Trace Material Quantities. It is common for canine handlers to correlate odor strength with the mass of energetic used, but a strong, localized odor can still be produced from trace material quantities. The volume of the vapor plume decreases with smaller material surface area, but the plume concentration, especially near the boundary layer, can be high. We can use this property, coupled with real-time detection, to determine trace contamination locations. Figure 1(B) shows a chromatogram produced by passing a 2-µg drop-casted TNT spot under the sample inlet. In this case, a TNT vapor filament was not observed before the end of the experiment, but signal increased when the TNT spot is closer to the sample inlet, showing TNT spatial distribution on the surface.

Odor Exfiltration Points. Explosives are typically encountered as hidden or wrapped in a packaged material. The instrument was used to determine an odor exfiltration point from a packaged explosive. A sample of C4 wrapped in plastic was analyzed by monitoring cyclohexanone. Signal was observed to increase whenever the inlet passed over a hole in the plastic wrapper (Figure 1(C)). For canine training purposes, we can generalize this behavior to concealed explosives in which the strongest vapor signature should be where odor exfiltration is the strongest, not necessarily where the material is hidden.

Canine Training Support

The instrument was used to directly support in-service canine teams. Bulk charges and ammunition storage cans used by the team were analyzed for cross-contamination, and a new means to validate the training ground truth was developed based on the presence of the actual odor plume.

Cross Contamination of Canine Training Materials. MRM analysis results for bulk charges are summarized in Table 2, and for ammunition cans that house the training charges in Table S2. Based on the expected energetics in each bulk charge, we typically monitored DNT, NG, PETN, RDX, and TNT. SESI was used in most cases, while DBDI was used when RDX and PETN signal were expected. The expected energetics were observed in all bulk charges and most ammunition cans. We observed residual odors in the ammunition cans, which is expected because trace amounts of explosives can be left behind from the bulk charge, and vapors can condense on surfaces. Their presence highlights the importance of storing material separately to reduce cross-contamination.

Several instances of cross-contamination, such as PETN and NG in C4, were observed in the bulk charges (Table 2, Table S2). We speculate that these contaminants were introduced with material-handling,[40] perhaps as aerosolized explosive particles from a neighboring charge. Contamination can be problematic for canine training because it exposes canines to unintended odors, especially if the contaminant has a high vapor pressure. NG, for example, has a vapor pressure

~100,000x higher than RDX, the expected energetic in C4. We detected ~90 ppt of NG from this sample of C4, which would unintentionally expose canines to a scent cue that contains ~100x higher concentration of NG than RDX. With this sample of C4, it would be difficult to determine if canines are properly trained to recognize C4 signatures.
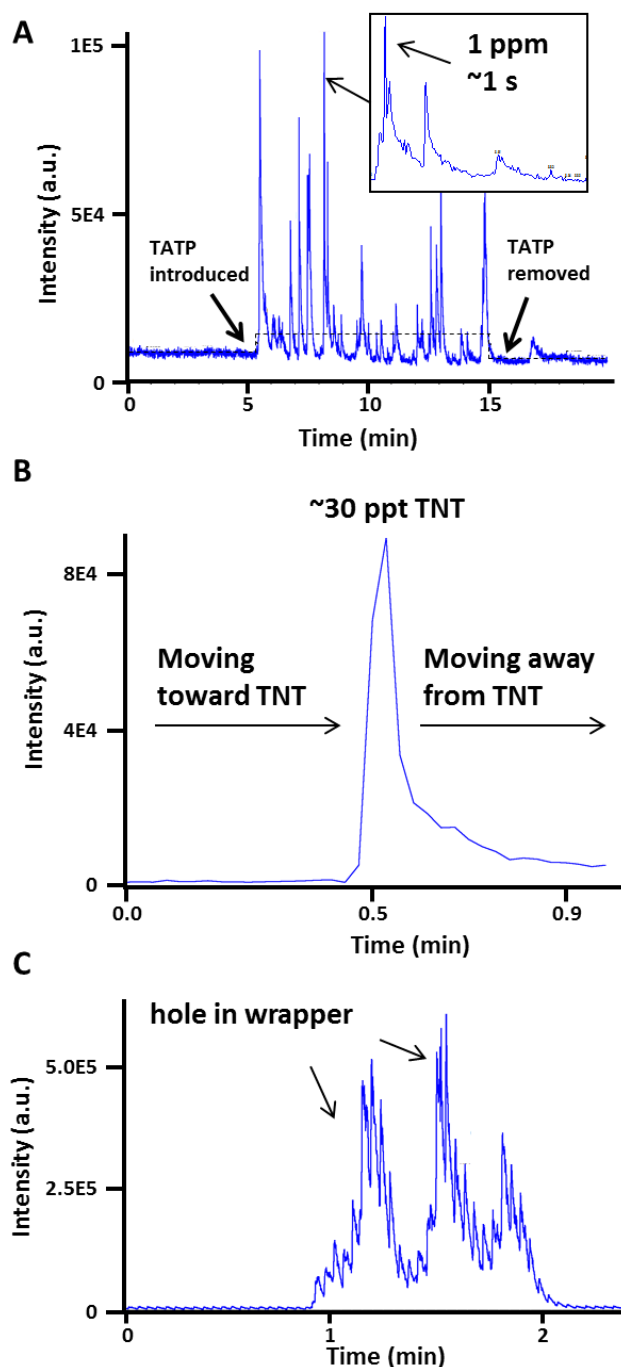

Figure 1. Plume dynamics and detection with real-time MS analysis. A. Signal from TATP placed 30 cm away is observed as pulses on a seconds time scale (inset) rather than as a constant, averaged signal (dotted line). B. Concentrated TNT vapor can be observed from trace TNT quantities. TNT signal increases when the source inlet approaches the TNT spot (~2 mm diameter). Distance traveled to the spot is ~50 cm. Total distance traveled ~100 cm. C. Stronger cyclohexanone signal

is observed from a hole in the C4 plastic wrapper, showing odor exfiltration points.

Q1 scans were also taken in addition to MRM and revealed multiple unidentified compounds (Table S3), suggesting that many odorants in addition to the energetics are present. Some of these chemicals are detected multiple times and may be related to plasticizers, solvents, or even common contaminants. Other possibilities include compounds from nitrile gloves or human skin, so canines may be recognizing signature from the handler rather than the explosives. These signatures have remained unidentified as of now. A detailed analysis will produce additional insights on odor cues in the canine's environment but is beyond the scope of this study.

Table 2. Energetics detected in bulk charges. Vapor concentrations are shown in parenthesis for each energetic.

	Expected Energetics (ppt)	Contaminant Energetics (ppt)
Ammonium Dynamite	NG (200)	-
TNT	TNT (20) DNT (30)[a]	-
C4	RDX (<1)	PETN (3) NG (90)
Smokeless Powder	DNT (40)[a]	TNT (2)
Detonation Cord	PETN (7)	-
Semtex-A	RDX (<1) PETN (4)	TNT (3)

[a] Assumes only 2,4-DNT as an isomer of DNT

Canine Performance in Training and Testing. Canine performance is traditionally scored against the handler's intention of whether or not an explosive is present at the indicated location. However, we believe that scoring is better conducted against whether or not a vapor plume is present. As we have shown, it is easy to expose canines to unintended odors through cross-contamination. Prepared trace energetics may also sublime away before canines have a chance to encounter the material.[41,42] Analyzing the vapor plume is a more direct verification of the canine's odor environment and should provide better performance evaluation. We term the difference as scoring against the ground truth (intended explosive is present), or against the vapor truth (vapor plume is present).

We held a canine testing exercise in which each canine had to find bulk, trace, and concealed explosives hidden both randomly and among indicated arrays. Table S4 breaks down canine performance based on the ground truth and the vapor truth. First a caveat, because performance data for in-service canines is considered sensitive, we cannot show actual performance values but instead present them using color scales. Further, not all blanks were analyzed due to time constraints. In these instances, we assumed agreement between the ground and vapor truths. We observed large performance variability between canines overall, consistent with prior studies.[2,3,5] Of note, the poor performance of canines 3 and 4 (Table S4) was attributed to physical exhaustion, as these two canines were deployed around the time of the experiment.

Several discrepancies were observed when comparing performance between the ground and vapor truths, which are shown in Table S4 as a + or − in the vapor truth columns. A plus (+) indicates that performance improved when evaluated via the vapor truth, and a minus (-) indicates decreased performance when evaluated via the vapor truth. Discrepancies manifested themselves as apparent canine mistakes that were later corrected by vapor analysis. As an example, Figure S4 shows the MRM spectrum of an empty envelope (i.e. blank) contaminated with RDX, effectively turning it in to an explosive hide. Table 3 summarizes the number of discrepancies observed between the two truths. Explosives vapor was detected in 27 out of 28 explosive hides, and contamination was detected in 6 out of 68 blanks. The two truths agree in 89 out of 96 instances (93%). It is important to note that most of the hides and blanks were prepared in a laboratory environment. Agreement may decline when preparation is done in field situations, especially for exercises conducted over many days involving multiple canine teams. Without vapor analysis, it would be difficult to differentiate canine mistakes from deficiencies in the training set up. Information revealed about the canine's odor environment can therefore improve performance evaluation accuracy.

Table 3. Ground truth versus the vapor truth in a canine testing scenario.

		Vapor Truth	
		Vapor Present	Vapor Absent
Ground Truth	**Hide**	27	1
	Blank	6	62

Beyond improved scoring, vapor analysis also provides the capability to correlate performance against odor strength, improving on the binary information in the ground truth (i.e. explosive is present or not). Unsurprisingly, we observed decreasing performance with lower vapor concentrations (Table S5). Existing training typically rates test difficulties based on the mass of the hidden explosives. We have shown that trace quantities can still produce a strong odor, especially near the material boundary layer (see "Plume Dynamics and Detection"). As an extension, a large explosive may be concealed to an extent where the exposed odor is weaker than odor from a trace amount of unconcealed explosive. The vapor strength, not the mass of explosives, should be a criterion for determining search difficulty, and vapor analysis may in the future be used to produce canine scent detection receiver operating characteristics curves (ROC).[11]

HMTD Training Aid Characterization

In addition to bulk explosives, some operational canine teams desire to use non-explosive training aids to serve as a safe surrogate for bulk explosives. Training aids are made by dispersing trace amounts of energetics onto an inert substrate, creating a product that produces odor without bulk quantities. There are currently no agreed upon standard for training aids, as there is still no consensus regarding the actual signaling compounds in explosives that cue canines. However, careful analysis can be used to begin standardizing training aid manufacturing to improve odor production consistency. The developed instrument has been used to help develop an HMTD

training aid, which is challenging because HMTD has a low vapor pressure and is susceptible to thermal degradation.[8-10] The developed mass spectrometer has good intensity linearity for HMTD (R^2: 0.996), low analysis temperature compared to GC instruments commonly used for quality control, and does not time average, generating time dynamics data that better mimic canine perception of the aid.

Based on previous work, HMTD and associated degradation products and starting materials were monitored in positive and negative polarities (Figure S3).[8,10] Measurements were taken over a two-hour period by sequentially alternating between positive and negative polarities. Two example data traces are shown in Figure S5. Figure S5(A) shows an aid with stable signal over the measurement period. Figure S5(B) shows an aid with undetectable HMTD signal and stronger signal for DMF, FA, and TMA compared to Figure S5(A). DMF signal was observed to decrease over time, suggesting that the vapor was trapped initially and was being lost to the surrounding. FA is a decomposition product of HMTD, and further autocatalyzes HMTD degradation, making FA a key compound to monitor and correlate with HMTD training aid quality.[10] From Figure S5(B), we show that even a badly degraded training aid can present several odors.

Beyond qualitative observations, we also aimed to develop a scoring method for judging training aid quality. The initial and final median concentrations of the six odorants (Figure S3) were used to calculate the 12-dimensional Euclidian distance between each training aid and the median signal from replicate measurements of pure HMTD controls. Figure 2(A) shows the distances (i.e. scores) for two types of training aids compared to pure HMTD: those made by distributing HMTD onto inert microfiber, and those made by using an inert powder support (Figure S6). The smaller the distance, the more similar the aid is to our pure HMTD controls. The large score range (~10-10,000) illustrates the degree of variability between aids, highlighting the need to be mindful of their composition and storage conditions. The analyzed fiber aids generally had headspace compositions different than pure HMTD, while some powder aids had more similar profiles. For aids with repeat measurements, we observed a trend of decreasing distance (increasing similarity) when aids were unsealed and stored uncapped in a fume hood (Figure 2(B)). This pattern is observed for some aids that were even sealed for 2, 6, and 12 months. For these HMTD training aids, we speculate that a sealed headspace accumulates degradation products. After removal from packaging, the accumulated decomposition vapors can escape when uncapped. Our scoring methodology may be improved by giving certain compounds preferential weighing factors, perhaps reflecting ionization efficiency or giving more importance to compounds deemed important for canine olfactory perception. However, the current scoring methodology is a simple way to judge training aid quality. It reveals that a proper storage protocol is important, and encouragingly, even poorly stored HMTD training aids may be improved by unsealing them.
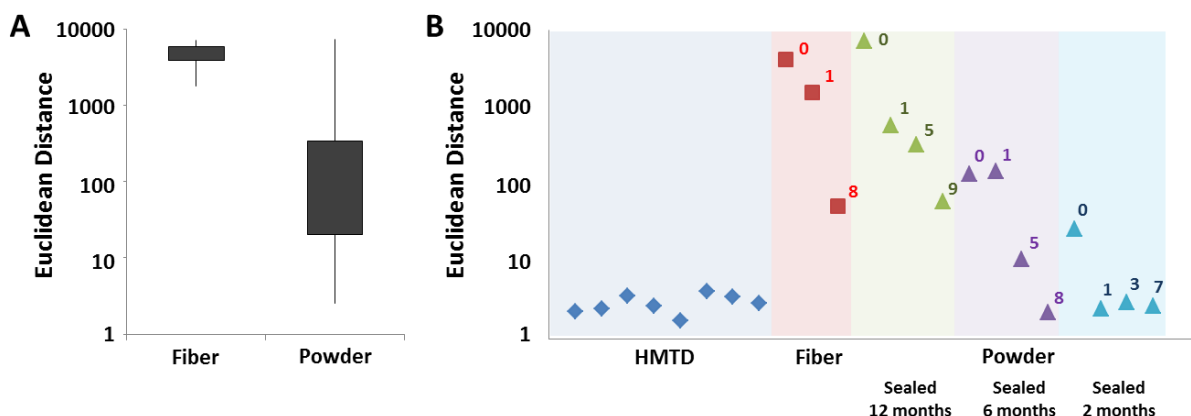

Figure 2. Summarizing score comparing HMTD training aids against pure HMTD. A. Training aid quality can be estimated by calculating the Euclidean distance between each training aid and the median of replicate pure HMTD measurements. The fiber training aids are generally unacceptable, while some powder training aids have profiles similar to pure HMTD. Fences indicate the minimum and maximum distance for each category. B. Training aids often become better mimics for pure HMTD after being stored uncapped to the storage environment. The number next to each data point indicates how many days the training aid was stored.

CONCLUSION

Current canine training does not use the same truth-validation and statistical rigor found in many scientific fields for sensor development. This issue is in part due to the difficulty of measuring a canine's odor environment. A vapor analysis mass spectrometer with real-time detection capability was therefore developed to support canine training. The instrument has a detection library relevant to canine training and can show aspects of vapor plume dynamics, such as plume filaments and odor exfiltration points. Analysis of canine training materials shows that it is easy to unintentionally expose canines to odors through cross-contamination, and improved evaluation is possible by using the vapor truth instead of the ground truth. Support was also provided for producing an HMTD training aid, monitoring the headspace for degradation products and starting materials. With sensitive, real-time vapor analysis, we can better evaluate canine performance and treat them as scientific chemical sensors in addition to service animals. The stronger training and validation rigor will improve the capability of the canine teams around the country.

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Supporting Information

Mass Spectrometry Vapor Analysis for Improving Canine Explosives Detection

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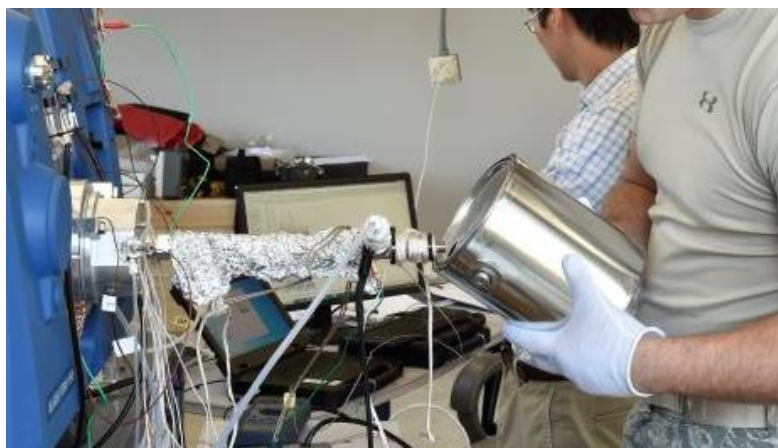ta-hsuan.ong@ll.mit.edu

Figure S-1. The developed vapor analysis mass spectrometer was used for in-field analysis of canine training materials.
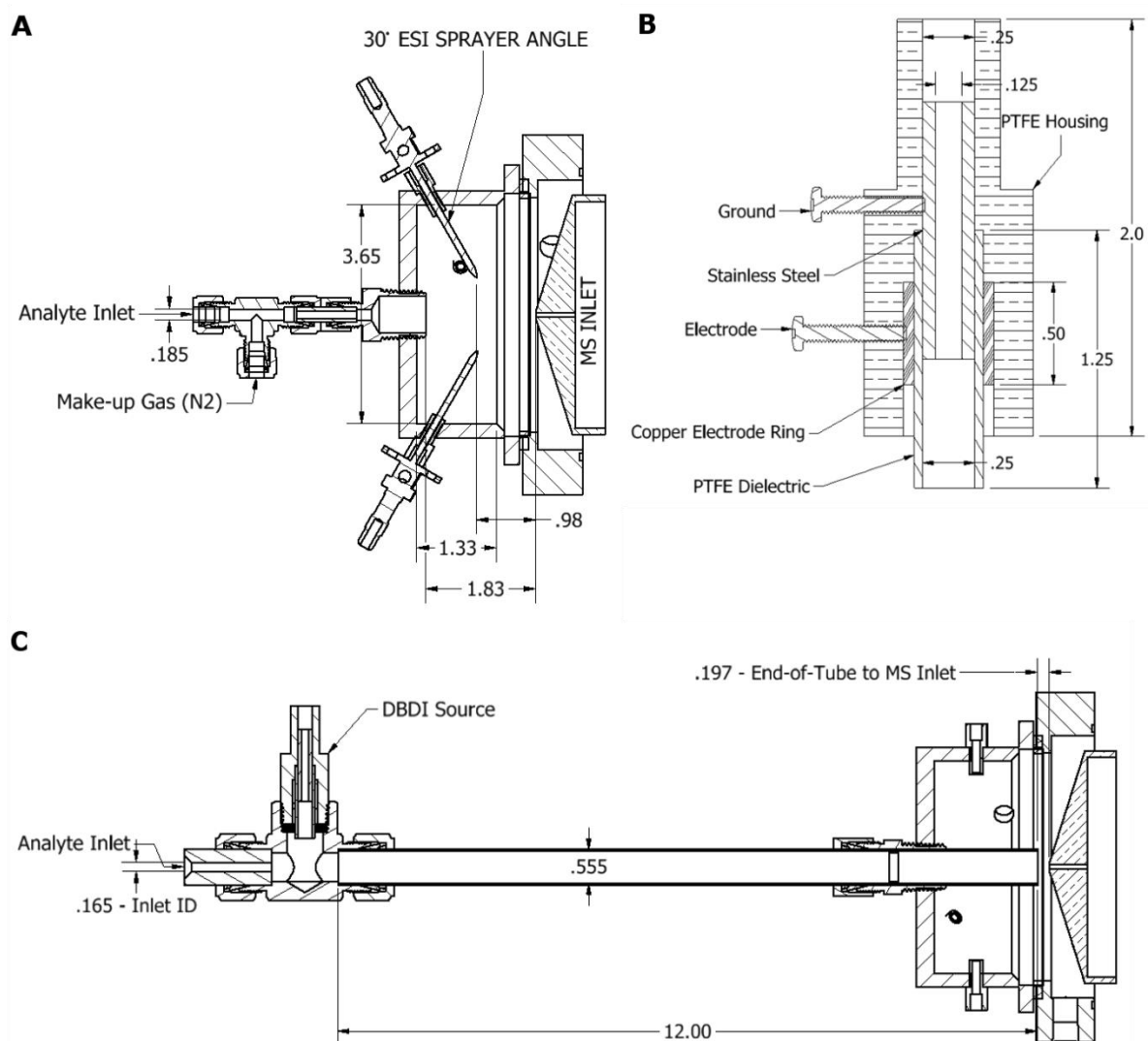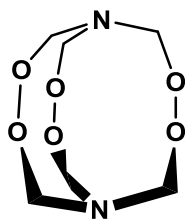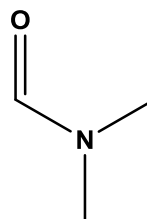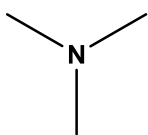
Figure S-2. Ionization sources for vapor detection of several energetics. All measurements are in inches. A. SESI source that uses separate ESI needles for positive and negative polarity analysis. B. DBDI assembly that produces a plasma. C. The DBDI assembly is placed perpendicularly to the analyte gas stream, decoupling plasma generation from sample analysis. The sample and reactive species from the plasma interact while traveling through a 1' ionization tube toward the mass spectrometer.

3

Hexamethylene Triperoxide Diamine
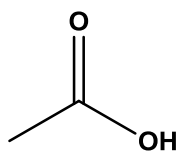**(HMTD)**
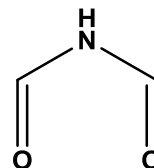**MRM Transition: 209/88**

Dimethyl Formamide
(DMF)
MRM Transition: 74/46

Trimethylamine
(TMA)
MRM Transition: 60/44

Hexamine
MRM Transition: 141/42

Formic Acid
(FA)
MRM Transition: 91/45

N-Formylformamide
(NFF)
MRM Transition: 72/42

Figure S-3. HMTD and several associated compounds were monitored in the headspace of HMTD training aids. Analysis covers positive and negative polarities. Masses for the most sensitive MRM transitions are shown.
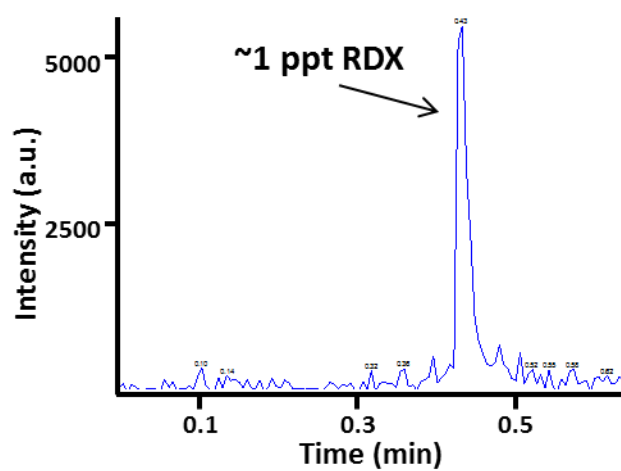
Figure S-4 RDX (~1 ppt) is detected from an empty envelope, turning a blank (ground truth) in to a hide (vapor truth).
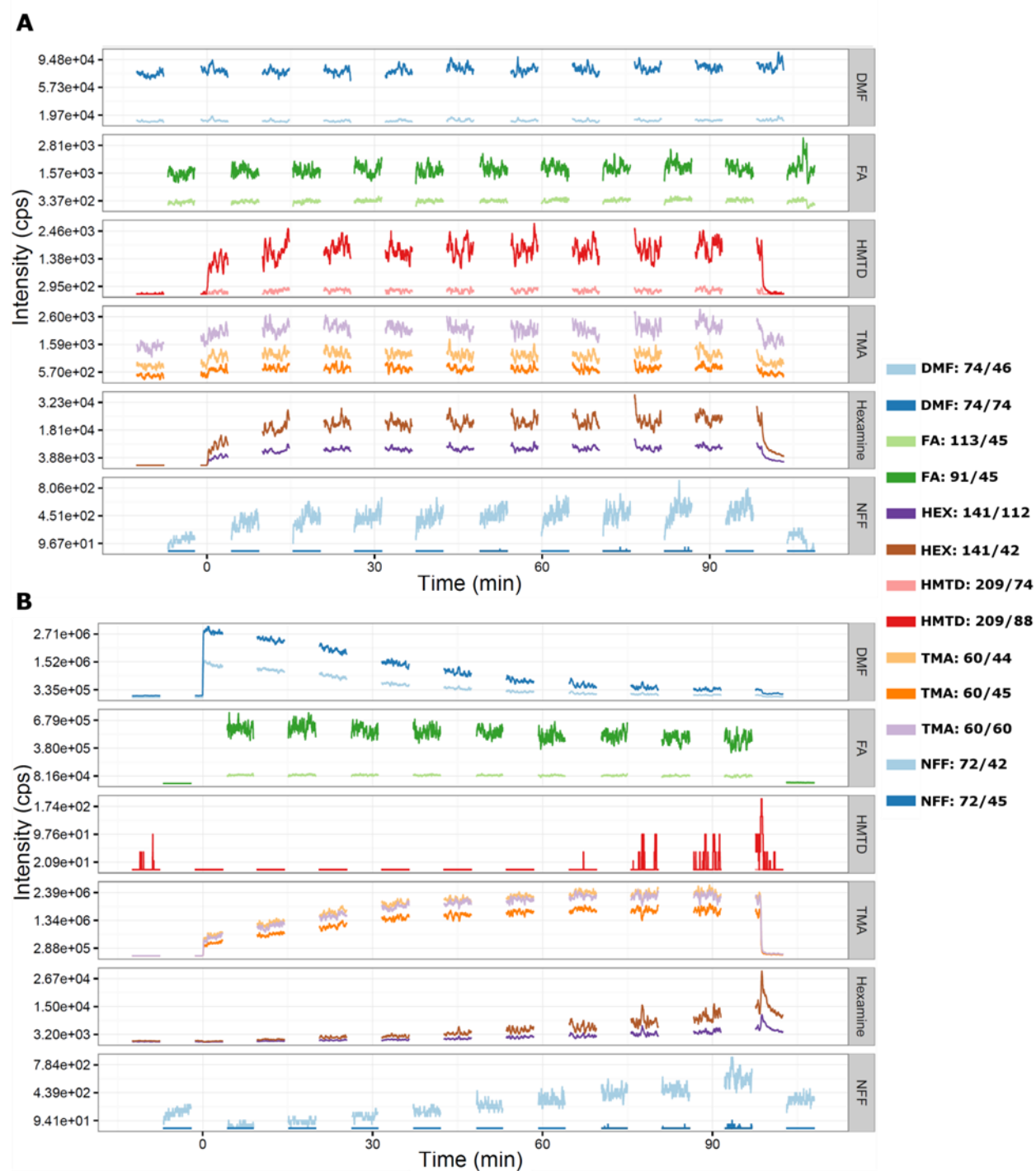
Figure S-5. Two example HMTD training aid measurement traces. A. A training aid with stable signal over the two hour analysis period. B. A training aid with strong formic acid signal and low HMTD signal. DMF signal decreases over time to near baseline, suggesting trapped vapor that is released to the surrounding. DMF: dimethyl foramide, FA: formic acid. HEX: hexamine. HMTD: hexamethylene triperoxide diamine, TMA: trimethyl amine, NFF: N-formyl formamide
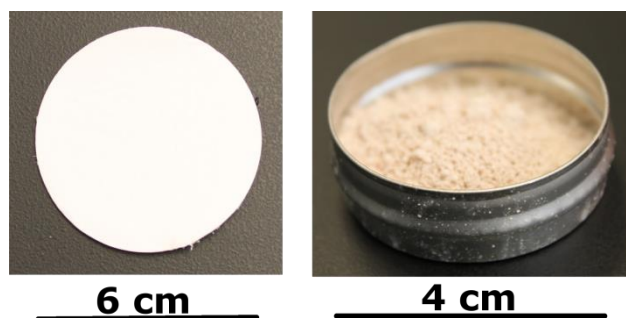
Figure S-6. Training aids were made by distributing HMTD onto inert microfiber or onto powder support.

Table S-1. Expected energetics component in bulk charges.

Bulk Charge	**Expected Energetics Component**
Ammonium Dynamite	NG
TNT	TNT, DNT
C4	RDX
Smokeless Powder	Nitrocellulose, DNT
Detonation Cord	PETN
Semtex-A	PETN, RDX

Table S-2. Energetics detected in ammunition cans for housing bulk charges. Vapor concentrations are shown in parenthesis for each energetic.

	Expected Energetics (ppt)	Contaminant Energetics (ppt)
Ammonium Dynamite	NG (8)	TNT (<1) PETN (<1)
TNT	DNT (8)[a] TNT (30)	-
C4	-	DNT (<1)[a]
Smokeless Powder	DNT (5)[a]	TNT (<1)
Detonation Cord	-	-
Semtex-A	-	-

[a] Assumes only 2,4-DNT as an isomer of DNT

TABLE S-3. Ions (*m/z*) detected in Q1 analysis of training materials

	Bulk Charge	Ammunition Can
Ammonium Dynamite	85.8, 211.9, 285.8	85.8, 166.2, 193.9, 195.9, 210.9, 211.9, 218, 225.9, 227.2, 240.9, 243.9, 262.1, 271.9, 272.9, 285.9, 288.1, 288.9
TNT	100	85.0, 99.9, 165.8, 184.1, 211.0, 225.9
C4	100, 179.1, 194	99.9, 115.9, 130.9, 131.9, 165.9, 173.3, 174.2, 179, 184.2, 194.1, 211.0, 235.0
Smokeless Powder	170.3	99.9, 211.0
Detonation Cord	None Detected	99.9
Semtex-A	100.0, 194.2	158.3, 184.1, 194.1, 235.1

TABLE S-4. Summary statistics of canine performance

	Ground Truth				Vapor Truth			
	Animal				**Animal**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
TN (%)	Green	Green	Green	Green	+	-	+	NC
FP (%)	Green	Green	Green	Green	+	-	+	NC
TP (%)	Yellow	Green	Red	Red	+	-	+	-
FN (%)	Yellow	Green	Red	Red	+	-	+	-

TN: True Negative; FP: False Positive; TP: True Positive; FN: False Negative

Green: TN, TP > 66%; FP, FN < 33%;

Yellow: 33% $\leq$ TN, TP, FP, FN $\leq$ 66%;

Red: TN, TP < 33%; FP, FN > 66%

NC: no change; +: improves compares to ground truth; -: worsens compares to ground truth

TABLE S-5. Canines show decreasing probability of detection (PD) with lower vapor concentrations.

	High (> 100 ppt)	Medium (< 100 ppt, > 1 ppt)	Low (< 1 ppt)
Performance	🟩	🟨	🟨

Green: PD > 66%

Yellow: PD ≤ 66%